THE RELATION OF HOMOZYGOUS DEFICIENCIES TO MUTATIONS AND ALLELIC SERIES IN MAIZE

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IN PREVIOUS investigations (McCLINTOCK 1938, 1941b), the author has presented evidence that homozygous minute deficiencies of specific regions of chromosomes in maize are responsible for the appearance of readily recognizable modified phenotypes. These phenotypes resemble recessive mutations in their expression. One of them exactly simulated and was allelic to a known recessive mutant (*bm1*). The evidence obtained from these investigations strongly suggests that one type of mutation process in maize is induced by loss of a minute segment of a chromosome which, when homozygous, produces a distinct phenotypic expression. The present investigation both supports and elaborates this contention in the following way. A particular minute segment was lost from the tip of the short arm of chromosome 9. In plants that were heterozygous for the deficient chromosome, the gametophytes and gametes possessing the deficient chromosome were completely functional. Upon self-pollination of such plants, normal appearing kernels were obtained that were homozygous for the deficiency. The homozygous deficient seedlings arising from these kernels were specifically modified in their phenotypic expression. They were pale-yellow. The pale-yellow mutant, although caused by a homozygous deficiency, is comparable in its genetic behavior to any typical recessive mutant. Its mendelian ratio, its "locus" in the chromosome and its linkage with other known mutants in the chromosome are strictly orthodox. If the presence of the deficiency were not known, the mutation would receive the same consideration as a "gene mutation." This relationship between deficiency and mutation may be elaborated further. This same segment plus an additional adjacent segment was removed from the chromosome. Even when this longer deficiency is present, the male and female gametophytes and gametes are viable and functional. Thus, individuals homozygous for this deficiency may be obtained. These individuals, in turn, show a phenotypic modification (white seedling) distinguishable from that produced by the shorter deficiency. When the two deficient chromosomes are combined in a zygote, the resulting seedling shows the pale-yellow phenotype associated with the shorter of the two deficiencies. In other words, the two mutants are allelic. The mutant produced by the shorter deficiency is dominant over the mutant produced by the longer deficiency. This might be expected, for the individuals possessing these two deficient chromosomes are homozygous deficient for only the shorter of the two deficiencies, that is the deficiency which produces the pale-yellow phenotype. Combinations of these two deficiency mutants with the previously known recessive mutant *yg2* (yellow-green plants; mutant located near the tip of the short arm of chromosome 9) have shown that *yg2* is allelic to and dominant

over the mutant produced by the longer deficiency (the white seedling mutant) but is non-allelic to the mutant produced by the shorter deficiency (the pale-yellow mutant). It is the purpose of this paper to show that the pale-yellow and white mutants are caused by specific homozygous deficiencies and to clarify the seemingly anomalous allelic relationships.

The method which produces these specific deficiencies is relatively simple. Thus, similar deficiencies and consequently similar mutations may be independently and repeatedly obtained.

THE METHOD OF OBTAINING TERMINAL DEFICIENCIES OF THE SHORT ARM OF CHROMOSOME 9

The deficiency mutants pale-yellow and white seedlings are associated with losses of terminal segments of the short arm of chromosome 9. The method by which terminal deficiencies arise has been described elsewhere (McCLINTOCK 1941a). It may be summarized briefly. Plants which possess a normal chromosome 9 and either a special rearrangement of segments of chromosome 9 or a chromosome 9 with a duplication of the short arm, can produce a dicentric chromatid at a meiotic prophase following specific types of crossing over between the two chromosomes 9. This dicentric chromatid produces a bridge configuration at one of the meiotic mitoses. Following breakage of this bridge, a chromosome 9 with a broken end will enter a spore nucleus. Depending upon where the break occurred within the bridge configuration, this chromosome 9 will be normal in chromatin constitution or will possess either a duplication or a deficiency of the short arm. All spores, except those with the very longest deficiencies, continue to develop. Because the two sister halves of the meiotically broken chromatid are fused at the position of previous breakage, a chromatin bridge is produced at the first spore anaphase as the two centromeres of the dicentric chromatid pass to opposite poles in the spindle figure. This bridge, in turn, will be broken and a newly broken chromatid will enter each telophase nucleus. The original meiotically broken chromosome 9 will continue this breakage-fusion-bridge cycle in the successive gametophytic divisions and also in the successive endosperm divisions whenever such a recently broken chromosome is introduced into the primary endosperm nucleus. However, when a chromosome 9 with such a recently broken end is introduced into the zygote by one of the gametes, the breakage-fusion-bridge cycle usually ceases in the young embryo. The broken end permanently heals and no longer participates in any fusions. The subsequent mitotic behavior of the chromosome 9 with a broken end is similar to that of any normal chromosome. In relatively infrequent cases, the chromosome with the broken end may continue the breakage-fusion-bridge cycle in the young embryo and even into the later developing sporophytic tissues.

When a zygote receives a normal chromosome 9 from one gamete and a recently broken chromosome 9 from the second gamete, the plants arising from these zygotes will have one normal chromosome 9 and, most frequently, one chromosome 9 with a broken but permanently healed end. The chromatin

constitution of this latter chromosome may be one of various types. This is because of its previous history of having been broken at meiosis and then having undergone the breakage-fusion-bridge cycle during the mitoses from meiosis to embryo formation. This chromosome may be normal in constitution or it may possess a duplication or a complex reduplication of segments of the short arm; or, terminal deficiencies or deficiencies plus duplications of segments may be the consequence of this behavior. Kernels which possess such a recently broken chromosome 9 may be identified by genetic means (for details, see McCLINTOCK 1941a). The chromatin constitution of the broken chromosome 9 in a plant arising from such a kernel can be determined by examination of pachytene configurations in this plant. The chromatin constitution of the broken chromosome 9 has been determined in over 500 plants which have arisen from such kernels. These plants were classified according to the observed modification in the constitution of the short arm of the broken chromosome 9. In all cases, the broken chromosome 9 contributed by one parent carried a dominant genetic marker in the short arm (*C*, aleurone color) whereas the normal chromosome 9 contributed by the other parent carried the recessive allele (*c*, colorless aleurone). The factor *C* is located in a normal chromosome 9 approximately one-third the distance from the end of the short arm. Although in a plant with unmodified chromosomes 9, more than 20 percent crossing over may occur between the locus of *C* and the end of the arm, a disturbed ratio of *C* to *c* could be expected following self-pollination of those plants which possessed a broken chromosome 9 with a decided modification of the short arm, such as a duplication or a deficiency. This is because the gametophytes with the modified chromosome carrying *C* might fail to function or might be reduced in functional capacity. Although all plants were self-pollinated to obtain this preliminary information on gametophytic functioning, our attention will be confined to (1) those that were classified as having received a broken chromosome 9 which is approximately normal, and (2) those which received a broken chromosome 9 with a deficiency. The extent of the deficiencies ranged from minute to extensive. All plants classified by pachytene studies as having a broken chromosome 9 with approximately a normal chromatin constitution gave normal ratios for aleurone color following self-pollination. Those classified as having received a chromosome 9 deficient for approximately one to six terminal chromomeres gave aleurone ratios suggesting that transmission of the deficient chromosome through the pollen did not occur, although transmissions through the female gametophyte were either normal or nearly so. (Six terminal chromomeres represent approximately the distal third of the short arm.) This was verified for each deficiency in later and more exacting tests. The majority of those plants classified as having a broken chromosome 9 with a very short terminal deficiency gave little or no evidence from the aleurone ratios of lack of functioning of either eggs or pollen grains carrying the deficient chromosome. It was presumed, therefore, that endosperms and embryos were being formed which were homozygous deficient for small terminal segments of the short arm of chromosome 9. From the

morphological appearance of endosperms or embryos, no distinction could be made, in many cases, between those kernels which were either normal or heterozygous for the deficiency and those which were homozygous for the deficiency.

To determine whether embryos with these homozygous deficiencies would germinate and produce viable seedlings, kernels from these self-pollinated ears were sown. For comparison, kernels from the self-pollinated ears of 30 plants classified as having a newly broken chromosome 9 with no deficiency were sown. All the seedlings arising from this latter group appeared normal. The plants arising from these seedlings were likewise normal in appearance. Some of these plants were examined at pachytene for their chromosome 9 constitutions. Two broken chromosomes 9 were present in some of these plants, indicating that no obvious phenotypic effects were being produced in plants that were homozygous for these broken chromosomes 9. In contrast, a segregation for seedling types occurred in the progeny of self-pollinated plants heterozygous for some of the small deficiencies. The modified seedlings in any one culture were either all pale-yellow or all white and in each culture, the ratio of these seedlings to the normal seedlings suggested a simple recessive mutation. In each case, linkage of the modified seedling type with the dominant aleurone factor *C* was clearly evident, indicating that the seedling character was associated with the deficient chromosome 9. This evidence suggested that the mutant seedlings, pale-yellow and white, might be produced when the chromosome complement was deficient for a small terminal segment of the short arm of chromosome 9. The following two sections of this paper will elaborate the methods used to verify this association. Seven independent cultures segregating pale-yellow seedlings and six independent cultures segregating white seedlings were selected for intensive study.

THE PALE-YELLOW SEEDLING MUTANTS

The pale-yellow seedlings in all seven cultures were very much alike in appearance. The seedlings appear to be normal in morphological characters and in growth rates. Although chlorophyll is present in the coleoptile, which is light green in color, the leaves show only a light yellowish color. These seedlings die following depletion of essential nutritive reserves in the kernels. The seven independently arising pale-yellow mutants will be referred to as *pyd* 1 to 7, respectively. (This symbolization implies a pale-yellow phenotype, produced by a deficiency.)

From a purely genetic standpoint, the *pyd* mutants may be treated as any other recessive mutant in maize. Typical ratios of 3 normal green seedlings to 1 pale-yellow seedling appear in the selfed progeny of plants heterozygous for any one of the *pyd* mutants (table 1). Linkage with *C* is shown in table 2. Linkage with *yg2* (yellow-green plants), which is known to be located near the end of the short arm of chromosome 9 (CREIGHTON 1934, MCCLINTOCK 1941a), must be very close; tests of 115 chromosomes derived from plants which carried *Pyd yg2* in one chromosome and *pyd Yg2* in the homologous chromosome gave

TABLE 1

The numbers of green and pale-yellow seedlings which appeared in the progenies of self-pollinated plants heterozygous for a deficient chromosome 9

SOURCE OF THE DEFICIENT CHROMOSOME	GREEN SEEDLINGS	PALE-YELLOW SEEDLINGS
*pyd*1	1411	445
*pyd*2	3404	1148
*pyd*3	1214	408
*pyd*4	747	253
*pyd*5	1565	545
*pyd*6	1114	400
*pyd*7	1910	636

no chromosomes with *Pyd Yg*2 or *pyd yg*2. The reported amount of crossing over between *yg*2 and *C* is approximately 19 percent (EMERSON, BEADLE and FRASER 1935). Estimates from the F_2 ratios of table 2 show that the amount of crossing over between *pyd* and *C* is similar to that between *yg*2 and *C*, although considerable variation between the *pyd* cultures is obvious. This variation is not considered significant since wide variations occurred between individual progenies within each *pyd* culture. On purely genetic evidence alone, the *pyd* mutants would be located near the tip of the short arm of chromosome 9.

TABLE 2

F_2 progenies showing linkage of the pale-yellow phenotype with C. Constitution of the F_1: pyd C/Pyd c

pyd CULTURE	*C* KERNELS				*c* KERNELS				% RECOMBINATION
	NUMBER PLANTED	% GERMINATED	SEEDLINGS		NUMBER PLANTED	% GERMINATED	SEEDLINGS		
			GREEN	PALE-YELLOW			GREEN	PALE-YELLOW	
*pyd*1	991	97.8	654	316	361	96.1	338	9	16
*pyd*2	1151	95.8	739	364	418	90.9*	366	14	20
*pyd*3	1261	99.2	851	401	376	98.1	363	7	14
*pyd*4	287	98.2	183	99	101	73.2*	73	1	11
*pyd*5	1202	98.6	775	411	412	95.4	384	9	15
*pyd*6	719	96.9	476	221	228	86.4*	195	2	11
*pyd*7	776	95.8	504	240	284	94.7	260	9	18

* The mutant *sh* (shrunken endosperm) closely linked with *c* was segregating in some of these cultures. Lowered germination rates are often encountered when the kernels are homozygous *sh*.

Pachytene examinations of plants heterozygous for any one of the *pyd* mutants were likewise heterozygous for their chromosome 9 constitutions. In all cases, a normal chromosome 9 and a chromosome 9 with a deficiency of a minute terminal segment of the short arm were present. The short arm of a

normal chromosome 9 usually terminates in a knob composed of heterochromatin. This knob is joined to the first distinct chromomere by a relatively thin strand of stainable chromatin (see diagram a, fig. 2). The knob and this thin strand of chromatin are missing in the deficient chromosome of all plants heterozygous for a *pyd* mutant (see diagram c, fig. 2). It has been determined that plants which are homozygous deficient for only the knob are quite normal in appearance. Thus, the effective chromatin loss, associated with a mutation to *pyd*, is presumably confined to the segment which joins the knob and the first distinct chromomere (or to a particular minute region within this segment; see the Discussion). For each case, the exact extent of the deficiency could not be stated with certainty. The segments being examined are too small for such microscopic resolution. Whether a specific *pyd* deficiency includes a minute segment of the first terminal chromomere or whether a minute proximal segment of the strand joining this chromomere with the knob is present could not be determined. However, in all *pyd* cultures, there is no question of the presence of a terminal deficiency which includes most of the strand joining the first chromomere with the knob.

If the homozygous deficiency were responsible for the pale-yellow character, the surviving green seedlings in the progeny of a selfed heterozygous plant would be either homozygous for normal chromosomes 9 or heterozygous for the deficient chromosome 9. Cytological examination of the chromosome 9 constitutions of the surviving green plants showed only these two types. In turn,

TABLE 3

*The cytologically determined chromosome 9 constitutions of the green plants of the various pyd cultures together with the results of tests for the presence or absence of the pyd mutants in each plant**

pyd CULTURE	CHROMOSOME 9 CONSTITUTION OF TESTED PLANTS					
	TWO NORMAL CHROMOSOMES 9			ONE NORMAL AND ONE DEFICIENT CHROMOSOME 9		
	NUMBER OF PLANTS EXAMINED	SEGREGATED *pyd*	DID NOT SEGREGATE *pyd*	NUMBER OF PLANTS EXAMINED	SEGREGATED *pyd*	DID NOT SEGREGATE *pyd*
*pyd*1	2	0	2	15	15	0
*pyd*2	5	0	5	13	13	0
*pyd*3	10	0	10	14	14	0
*pyd*4	6	0	6	14	14	0
*pyd*5	4	0	4	18	18	0
*pyd*6	1	0	1	17	17	0
*pyd*7	6	0	6	26	26	0
Totals	34	0	34	117	117	0

* Presence of the *pyd* mutant detected by one or more of the following methods: selfing, crosses to plants heterozygous for long terminal deficiencies of the short arm of chromosome 9, sib-crosses and intercrosses to various plants heterozygous for *pyd* or *wd* mutants (see text for elaboration of these methods).

if these latter plants are appropriately tested for the presence of the *pyd* mutant in one of their chromosomes 9, only the plants heterozygous for the deficiency should give rise to pale-yellow seedlings whereas those having two normal chromosomes 9 should not segregate any pale-yellow seedlings. This was found to be true in all subsequent progenies of cultures carrying the pale-yellow mutants. This evidence is summarized in table 3. Among the 151 plants cytologically examined, the 34 which possessed two normal chromosomes 9 gave rise only to normal green seedlings whereas all of the 117 plants which possessed a normal and a deficient chromosome 9 segregated pale-yellow seedlings.

To obtain further evidence for the association of the pale-yellow phenotype with a homozygous deficient condition, pollen from heterozygous deficient plants of each of the seven *pyd* cultures was placed upon silks of plants heterozygous for only female transmissible terminal deficiencies of the short arm of chromosome 9. These terminal deficiencies ranged in length from one chromomere to six chromomeres. If *pyd* were associated with a minute, terminal, male and female transmissible deficiency, pale-yellow seedlings should appear in the progeny of all such crosses following zygotic combinations of the two deficient chromosomes 9. In no case would the deficient chromosome contributed by the female parent cover the deficiency in the chromosome contributed by the male parent. This proved to be true (table 4).

The similarity in phenotypic appearance and location in the chromosome, as shown by linkage relations with *yg2* and *C*, of all seven independently arising

TABLE 4

Phenotypic appearance of plants with the short terminal deficiency of the pyd and wd cultures and a longer terminal deficiency; py represents pale-yellow seedlings, w represents white seedlings

SOURCE OF DEFICIENT CHROMOSOME FROM ♂ PARENT	APPROXIMATE EXTENT OF TERMINAL DEFICIENCY OF THE SHORT ARM OF THE CHROMOSOME 9 CONTRIBUTED BY THE FEMALE PARENT				
	1 CHROMOMERE	2 CHROMOMERES	3 CHROMOMERES	4 CHROMOMERES	6 CHROMOMERES
*pyd*1	py		py		py
*pyd*2		py			
*pyd*3	py	py		py	py
*pyd*4	py	py		py	py
*pyd*5	py	py			py
*pyd*6	py	py			py
*pyd*7		py		py	py
*wd*1		w	w	w	w
*wd*2	w	w	w	w	w
*wd*3	w	w	w	w	w
*wd*4	w	w		w	w
*wd*5	w			w	w
*wd*6	w				w

pyd mutants, together with a similar extent of deficiency in the chromosome 9 associated with each mutant, suggested that all seven *pyd* mutants were the expression of one and the same causal condition. If this were so, then combinations of any two of the seven *pyd* mutants should produce the pale-yellow phenotype. Intercrosses between heterozygous deficient plants of all seven cultures were made. Pale-yellow seedlings segregated in the expected ratios in the

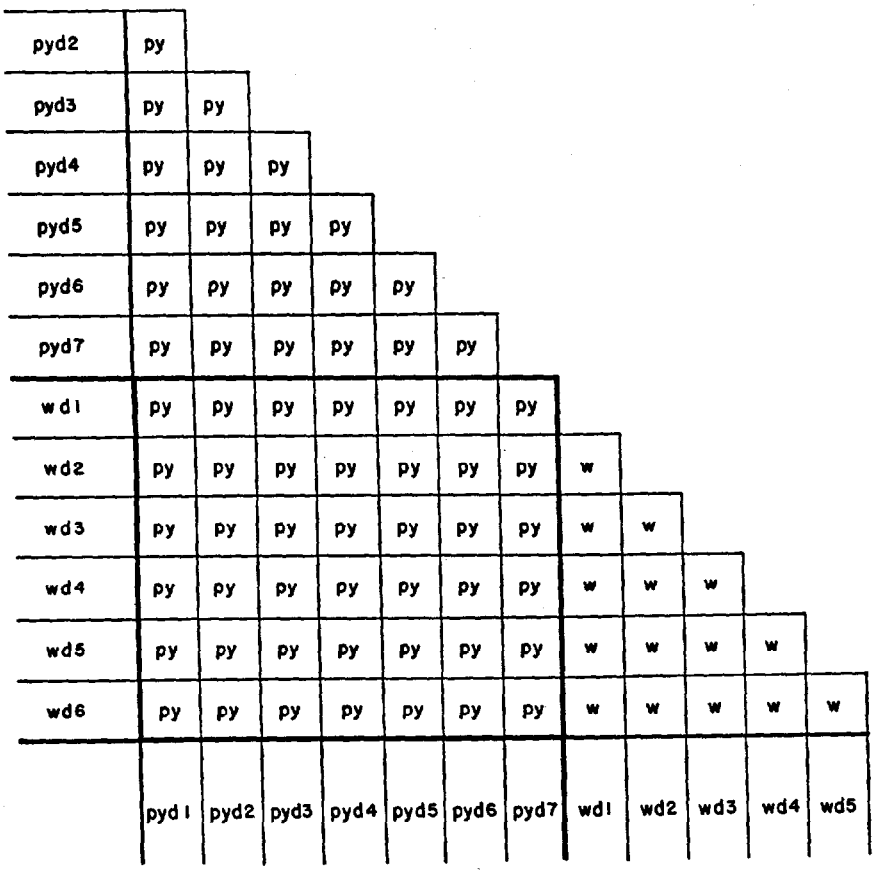


FIG. 1.—The phenotypic appearance of seedlings following combinations of all seven *pyd* mutants (upper triangle), of all six *wd* mutants (triangle to lower right) and of all seven *pyd* mutants with all six *wd* mutants (central rectangle). The symbols py and w in the small squares represent pale-yellow and white seedling phenotypes, respectively.

progeny of all 21 possible combinations (fig. 1). For economy of space, the ratios of green to pale-yellow seedlings in the progeny of the 21 combinations have not been included in tabular form. However, all gave typical 3:1 ratios. These crosses established the iso-allelic if not identical nature of all seven *pyd* mutants. (Iso-alleles are defined by STERN and SCHAEFFER (1943) as alleles indistinguishable except by special tests.)

THE WHITE SEEDLING MUTANTS

The six white seedling mutants are readily distinguishable from the pale-yellow mutants. The coleoptile in some cultures is very slightly tinged with yellow color whereas in other cultures it is chalk-white. The leaves are either chalk-white or slightly tinged with a very faint yellow color. Although the general morphological form of these white seedlings appears to be normal, they are always smaller than their sister green seedlings of the same age. The six white seedling mutants will be referred to as *wd*1 to *wd*6, respectively. This symbolization refers to the white phenotype produced by a deficiency.

The plants which segregate white seedlings in the six *wd* cultures are heterozygous for a terminal deficiency of the short arm of chromosome 9. These deficiencies are longer than those associated with the *pyd* mutants. They include not only the knob and the chromatin thread connecting the knob with the first distinct chromomere, as in the *pyd* mutants, but in addition a part of the first distinct chromomere is missing (see diagram d, fig. 2). In each case, it was not possible to determine the exact amount of terminal chromatin that was missing. However, the best preparations indicate that the deficiencies which cause the *wd* mutants extend to about the middle of the first chromomere.

The white seedlings in the progeny of self-pollinated heterozygous deficient plants die following depletion of essential nutritive reserves in the kernels. Cytological examination at pachytene of the chromatin constitution of the chromosomes 9 were confined, therefore, to the surviving green plants. Like

TABLE 5

*The cytologically determined chromosome 9 constitutions of the green plants of the various wd cultures together with the results of tests for the presence or absence of the wd mutant in each plant**

wd CULTURE	CHROMOSOME 9 CONSTITUTION OF TESTED PLANTS					
	TWO NORMAL CHROMOSOMES 9			ONE NORMAL AND ONE DEFICIENT CHROMOSOME 9		
	NUMBER OF PLANTS EXAMINED	SEGREGATED *wd*	DID NOT SEGREGATE *wd*	NUMBER OF PLANTS EXAMINED	SEGREGATED *wd*	DID NOT SEGREGATE *wd*
*wd*1	3	0	3	25	25	0
*wd*2	14	0	14	10	10	0
*wd*3	9	0	9	20	20	0
*wd*4	9	0	9	17	17	0
*wd*5	0	—	—	6	6	0
*wd*6	1	0	1	15	15	0
Totals	36	0	36	93	93	0

* See footnote, table 3.

the *pyd* mutant cultures, only plants which were homozygous for normal chromosomes 9 or heterozygous for the deficient chromosome 9 were found. These plants, in turn, were tested for segregations of white seedlings in their progeny. None of the 36 examined plants with two normal chromosomes 9 gave rise to white seedlings, whereas all of the 93 examined plants which were heterozygous for the deficiency gave rise to white seedlings (table 5). This is to be expected if the white seedling phenotype is caused by the homozygous deficiency.

It was emphasized that the deficiencies associated with the pale-yellow phenotypes gave none of the usual genetic evidences of the presence of a deficiency. Except for the changed chlorophyll condition, all other examined tissues, homozygous for the deficiency, appeared to be normal. In contrast, the homozygous deficiency associated with the white seedling condition reflects the presence of a deficiency in several ways. In the first place, the transmission of the deficient chromosome through the pollen in competition with pollen carrying a normal chromosome 9, is reduced in two of the six *wd* mutants (*wd2* and *wd6*). Indications of this were apparent from the ratios of *C* to *c* obtained following self-pollinations of the heterozygous deficient plants (*C* carried by the deficient chromosome; *c* carried by the normal chromosome) and following backcrosses of these plants to normal plants homozygous for *c* (table 6, I and II). The aleurone ratios obtained from similar crosses involving the other four *wd* cultures did not suggest such selective reduction in pollen func-

TABLE 6

I. Ratios of C to c following self-pollination of plants heterozygous for the deficient chromosomes 9 of the white-seedling cultures. C carried by the deficient chromosome, c carried by the normal chromosome.

II. Ratios of C to c obtained when the pollen of plants in I was placed upon silks of normal plants homozygous for c.

wd CULTURE	I		II	
	C	*c*	*C*	*c*
wd1	4296	1416	1126	1132
wd2	745	371	1455	1949
wd3	925	288	2781	2734
wd4	1761	588	1620	1549
wd5	387	276	1858	1781
wd6	2545	1146	2931	4883

tioning. More exact tests of the gametophytic transmissions (see page 491 and table 10) have shown that the transmission of the deficient chromosome through the female gametophyte is normal for all six white seedling-producing deficiencies and is normal through the pollen for *wd1*, 3, 4 and 5. However, in competition with normal pollen, the functioning of pollen carrying the deficient chromosome is reduced in the *wd2* and *wd6* cultures. The percentage reduction is approximately the same in each case. The pollen utilized had equal

numbers of normal and deficient grains. However, only one deficient pollen grain effected fertilization for every two normal grains. Although white seedlings appear when all six deficiencies are homozygous, it is to be expected from the method of origin that all six of these independently arising deficiencies need not be exactly alike in the extent of the deficiency (see Discussion). However, they all include the segment of chromatin which, when homozygous deficient, is responsible for the chlorophyll abnormality.

Examination of the kernels derived from self-pollinations of plants that are heterozygous for these deficiencies revealed another character which is consistent with a homozygous deficient condition. In all six cultures, some of the embryos had died during various stages of embryonic development. These embryos were shriveled and discolored and did not germinate. Kernels with such dead embryos could readily be classified. There was no consistency in the proportion of kernels with defective embryos among the self-pollinated ears

TABLE 7

Segregation of defective embryos among the C and c kernels derived from self-pollination of plants heterozygous for the deficient chromosomes 9 of the white seedling cultures. C carried by the deficient chromosome, c carried by the normal chromosome.

wd CULTURE	*C* KERNELS		*c* KERNELS	
	NORMAL EMBRYOS	DEFECTIVE EMBRYOS	NORMAL EMBRYOS	DEFECTIVE EMBRYOS
*wd*1	3553	743	1395	21
*wd*2	710	35	367	4
*wd*3	893	32	286	2
*wd*4	1617	144	562	26*
*wd*5	784	53	275	1
*wd*6	2245	300	1140	6

* Twenty-five of these kernels came from two of the six ears counted. Their cause is probably not related to the deficiency in chromosome 9.

within any white seedling culture. On some ears, no such embryos were present whereas on other ears they ranged from a few to approximately 25 percent of the embryos. Linkage of this defective embryo character with the mutant *C*, carried by the deficient chromosome 9, was obvious in all cases, suggesting that the cause of the defective embryo was associated with the deficiency (table 7).

In the progeny of self-pollinated heterozygous deficient plants, the typical F_2 ratio of 3 normal green seedlings to one white seedling is not always present. Sometimes there is a deficiency of the white seedling class. This would be expected if the homozygous deficiency causes death of some but not all of the developing embryos. Only those that survive during embryogeny could produce white seedlings. Lack of effective germination of some apparently living embryos which are homozygous deficient probably takes place for germination

rates were definitely reduced in some of these F_2 cultures. Also, within the *wd2* and *wd6* cultures, the reduced functioning of the pollen grains carrying the deficient chromosome 9 would tend to lower the percentage of homozygous deficient embryos and thus the proportion of white seedlings in the progeny. This latter factor, which reduces the expected proportion of white-seedlings in the F_2 progenies, is relatively constant whereas the former two factors are highly variable among the individual F_2 cultures.

Since wide variations in the proportion of normal to white seedlings occurred among the individual progeny tests within each white seedling culture, a composite table of these ratios for each of the white seedling cultures does not reveal the association of the reduction in the proportion of white seedlings with any one of the three mentioned causes. In a particular progeny, none, one, two or, in the *wd2* and *wd6* cultures, all three factors responsible for the reduc-

TABLE 8

F_2 progenies showing linkage of the wd mutants with C. Constitution of F_1: Deficient chromosome 9 with C/normal chromosome 9 with c.

wd culture	GOOD EMBRYOS								DEFECTIVE EMBRYOS (NO GERMINATION)		TOTAL SEEDLINGS	
	C KERNELS				*c* KERNELS							
	NUMBER PLANTED	% GERMINATED	SEEDLINGS		NUMBER PLANTED	% GERMINATED	SEEDLINGS		*C* KERNELS	*c* KERNELS	GREEN	WHITE
			GREEN	WHITE			GREEN	WHITE				
wd1	1631	89.5	1283	177	639	96.2	615	0	339	9	1898	177
wd2	420	86.4	303	60	196	83.6†	162	2	5	0	465	62
wd3	893	94.0	575	265	286	92.3	258	6	32	1	833	271
wd4	798	91.1	499	229	260	75.7†	191	6	72	25*	689	235
wd5	784	89.9	505	200	275	92.3	247	7	53	1	752	207
wd6	750	95.3	550	165	318	96.8	307	1	22	0	857	166

* See footnote, table 7.
† See footnote, table 2.

tion in the expected proportion of white seedlings may be active. This relationship is brought out in table 8 where the ratios for *C* and *c*, the proportion of defective embryos in each class, and the germination rates are considered. To illustrate how the three factors operate individually, the progenies from three selected ears in which only one factor was effectively operating in each case are given in table 9. In this table, a fourth progeny is added in which none of these factors was effectively operating. In this latter case, the expected ratio of 3 normal green to 1 white seedling is apparent.

The association of the white seedling character and the defective embryo condition with a homozygous deficient state can be verified by combining the deficient chromosomes of the white seedling cultures with the various female transmissible deficient chromosomes 9 given in table 4. Plants heterozygous for the female transmissible deficiencies of table 4 were crossed by plants heterozygous for the deficiencies of the six white seedling cultures. Some defective

TABLE 9

F_2 progenies from three individual ears illustrating the three factors which materially reduce the expected proportion of white seedlings; together with the progeny from a fourth ear in which none of these factors was operating. Constitution of F_1: Deficient chromosome 9 with C/normal chromosome 9 with c.

FACTOR OPERATING	ALEURONE RATIO		GOOD EMBRYOS								DEFECTIVE EMBRYOS (NO GERMINATION)		TOTAL SEEDLINGS	
			C KERNELS				*c* KERNELS							
			NUMBER PLANTED	% GERMINATED	SEEDLINGS		NUMBER PLANTED	% GERMINATED	SEEDLINGS					
	C	*c*			GREEN	WHITE			GREEN	WHITE	*C*	*c*	GREEN	WHITE
Reduced functioning of pollen with deficient chromosome (from *wd* 6 culture)	258	101	257	98.4	193	60	101	99.0	100	0	1	0	293	60
Defective embryos (from *wd*1 culture)	317	119	226	96.9	210	9	118	97.4	115	0	91	1	315	9
Poor germination (from *wd*1 culture)	333	113	325	76.9	196	54	111	91.9	102	0	8	2	298	54
No selective elimination (from *wd*3 culture)	314	111	312	98.7	200	108	111	97.3	106	2	2	0	306	110

embryos, showing linkage with *C*, appeared in many of these crosses. In all cases, white seedlings likewise appeared in the progeny (table 4). These results are comparable to the selfed progeny of heterozygous deficient plants within the various white seedling cultures. This could be expected because the gametic combination of the two deficient chromosomes 9 would give rise to an individual which is homozygous deficient for only the short terminal deficiency of the white seedling cultures. It seems clear, then, that both the defective embryo and white seedling character are the consequence of the particular homozygous deficient state.

As stated previously, *yg2* is known to be located close to the end of the short arm of chromosome 9. Combinations of the deficient chromosomes 9 of the white seedling cultures with a normal chromosome 9 carrying *yg2* proved to be illuminating. It will be recalled that in the *wd* cultures the surviving green plants in the progeny of a self-pollinated heterozygous deficient plant are of two types: (1) those with two normal chromosomes 9 and (2) those with a normal and a deficient chromosome 9. When 15 plants of the former type were crossed by plants homozygous for *yg2*, the progeny from all 15 crosses gave only normal green plants. In contrast, when heterozygous deficient plants [(2) above] from all six *wd* cultures were crossed by plants homozygous for *yg2*, normal green plants and yellow-green plants appeared in the F_1 progeny. The

ratios in each case (table 10) were those expected if the green plants resulted from the zygotic combination of the normal chromosome 9 from the heterozygous parent with the *yg2* carrying chromosome and if the yellow-green phenotype resulted from the combination of the deficient chromosome with the *yg2* carrying chromosome. Six of these yellow-green plants were examined at pachytene for their chromosome 9 constitutions. All possessed one normal chromosome 9 and the deficient chromosome 9 of the *wd* cultures. These and four other yellow-green plants not examined cytologically, were appropriately tested for the presence of the white seedling mutant. The progeny tests revealed the presence of the *wd* mutant in all ten cases. In turn, none of the 22

TABLE 10

Relative transmissions of the deficient and the normal chromosome through the ♀ and ♂ gametophytes of plants heterozygous for the various wd deficiencies. Tests made by reciprocal crosses of the heterozygous deficient plants to normal plants homozygous for yg2. The green plants in the progeny represent transmissions of the normal chromosome; the yellow-green plants represent transmissions of the deficient chromosome.

CULTURE	TRANSMISSIONS THROUGH THE ♀ GAMETOPHYTE		TRANSMISSIONS TNROUGH THE ♂ GAMETOPHYTE	
	GREEN	YELLOW-GREEN	GREEN	YELLOW-GREEN
wd1	964	917	406	442
wd2	614	609	675	319
wd3	167	173	800	761
wd4	969	977	809	811
wd5	602	611	654	675
wd6	454	439	1350	679

cytologically examined green plants possessed a deficient chromosome 9 and none of the green plants segregated white seedlings following appropriate tests. These results indicated that from the point of view of phenotypic expression, the *wd* mutants are allelic and recessive to *yg2*.

The *yg2* factor was not present in either of the chromosomes 9 of the plants which gave rise to the broken chromosomes. Therefore, it cannot be concluded that *yg2* was present in the original broken chromosome unless a mutation to *yg2* occurred during the formation of the white seedling-producing deficiencies. As stated previously, the seven *pyd* mutants were not allelic to *yg2*. During the formation of these seven deficiencies responsible for *pyd*, no mutation to yellow-green occurred. The allelic relationships of *yg2* and *wd* is best explained by considering that the deficiencies causing the *wd* mutants are long enough to include the *Yg2* locus. The presence of the yellow-green plants in these crosses is, thus, the expression of a hemizygous condition, no complementary locus of *yg2* being present in the deficient chromosomes 9 of the *wd* cultures.

The allelic relations of *yg2* and the *wd* mutants allowed a convenient means of determining the transmissions of the deficient chromosomes through the

male and female gametophytes for each of the six deficiencies. To determine the transmissions through the female gametophyte, heterozygous deficient plants of the six *wd* cultures were pollinated by plants homozygous for *yg2*. To determine the transmissions through the male gametophytes (pollen grains), the reciprocal crosses were made. Because there are no viability factors connected with embryo development of the yellow-green phenotype, the ratio of green to yellow-green seedlings is a direct measure of the transmissions of the normal and the deficient chromosomes, respectively. The results are given in table 10. The deficient and the normal chromosomes are equally transmitted through the male and female gametophytes of cultures *wd*1, 3, 4 and 5. Equal transmissions occur through the female gametophytes of cultures *wd2* and *wd*6 but the transmission of the deficient chromosome through the male gametophyte is definitely reduced. In both cases, approximately one-third instead of one-half of the progeny received the deficient chromosome. The allelic relationship of *yg2* and *wd* also allowed a determination to be made of the amount of crossing over which occurs between the mutant *C* and the end of the short arm of the deficient chromosome. Reciprocal crosses were made between normal chromosome 9 plants homozygous for *yg2* and *c* and heterozygous deficient plants carrying *Yg2* and *c* in their normal chromosome 9 and *C* in their deficient chromosome 9. The non-crossover chromatids would give rise to (1) colorless kernels, green plants and (2) colored kernels, yellow-green plants. The crossover chromatids would give rise to (1) colorless kernels, yellow-green plants and (2) colored kernels, green plants. Within each white seedling culture, wide variations in crossover percentages were found among the individual progenies. Considerably less variation occurred when the male was the heterogametic parent; the average for all six cultures was 15.2 percent, which is close to the 19 percent previously reported for *yg2* and *C*.

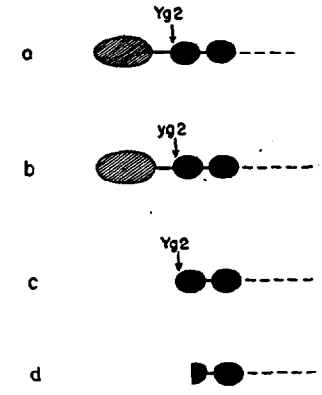
The similar appearance of the white seedling phenotypes, the presence of defective embryos, the allelic relations with *yg2* and the association with a terminal deficiency of all six white seedling mutants suggested that they might be either identical or iso-allelic. To determine this, intercrosses between heterozygous deficient plants of all six cultures were made. Both defective embryos and white seedlings showing linkage with *C* segregated in the F_1 progeny of all 15 combinations establishing, therefore, their iso-allelic if not identical nature (fig. 1).

The description so far given allows one to draw the following conclusions. The seedling mutant pale-yellow will appear whenever a plant is homozygous deficient for a small terminal segment composed of the knob and the chromatin strand joining the knob and the first distinct chromomere. All such mutants will be allelic if not identical. They will not be allelic to *yg2* but will be very closely linked with this locus. A white seedling mutant will appear whenever a plant is homozygous deficient for this same segment plus a particular part of the adjacent chromomere. All of these white seedling mutants will be either identical or allelic. In contrast to the pale-yellow mutants, all the white seedling mutants will be allelic and recessive to the mutant *yg2*. Following these

conclusions, one should expect that the combination of the deficient chromosome which produces pale-yellow seedlings and the deficient chromosome which produces white seedlings would give rise to the pale-yellow phenotype for these seedlings would be homozygous deficient for only the segment associated with the pale-yellow phenotype. To determine this, intercrosses of heterozygous deficient plants of all seven *pyd* cultures with heterozygous deficient plants of all six *wd* cultures were made. In all 42 combinations, pale-yellow seedlings segregated in the expected ratios in the F_1 progeny (fig. 1). Furthermore, there were no defective embryos regularly appearing in the progeny of these crosses. It is clear then, that the pale-yellow and white mutants are allelic and that the white mutants are recessive to the pale-yellow mutants.

GRAPHIC INTERPRETATION OF THE ALLELIC RELATIONS OF *pyd*, *wd* AND *yg2*.

A consistent hypothesis can be formulated to account for the appearance of the pale-yellow and white seedling mutants and their allelic relations with each other and with *yg2*. This hypothesis considers that the phenotypes *pyd* and *wd* are due to homozygous deficiencies, as elaborated in the previous sections. Likewise, it is possible that *yg2* may be due to or simulated by a homozygous minute internal deficiency. Whether or not *yg2* is due to a homozygous deficiency or a true "gene" mutation is immaterial, however, in the explanation of the allelic relations of these three mutants. To facilitate this interpretation, a diagram, figure 2, has been constructed. The short arm of a normal chromosome 9 carrying *Yg2* and terminating in a knob is shown in **a**, figure 2; **b**, figure 2, represents a normal chromosome 9 carrying *yg2*. In **a** and **b** the arrow points to the locus of *Yg2* and *yg2* respectively. In **c**, a terminal segment is missing. This is the segment which, when homozygous deficient, is responsible for the pale-yellow mutant. It should be noted that this deficient segment does not include the *Yg2* locus. In **d**, a longer terminal segment is missing. It is the segment which, when homozygous deficient, produces the white seedling mutants. It should be noted that this deficiency includes the locus of *Yg2*. Below and to the left of the diagram is given the phenotypes appearing when a plant is homozygous for any one of these chromosomes. To the right are given the phenotypes produced following combinations of any two of these chromosomes. The normal chromosome 9 with *Yg2* (**a**, fig. 2) covers the recessive mutant *yg2* of **b**, and the deficiencies of both chromosomes **c** and **d**. Thus, only green seedlings arise following combinations of this chromosome with any one of the other three. The combination of **b** plus **c** gives rise to a green seedling because the *yg2* carrying chromosome covers the deficiency in chromosome **c** whereas the deficient chromosome **c** carries the dominant allele of *yg2*. The combination of **c** and **d** gives rise to a pale-yellow seedling because the residual homozygous deficiency is only that which produces the pale-yellow phenotype. In the combination **b** plus **d**, however, the seedling is yellow-green because the terminal deficiency in chromsome **d** is covered by chromosome **b** but chromosome **d** does not cover the *yg2* locus with *Yg2* because it is deficient for this locus.



	Phenotype appearing when homozygous		Phenotype appearing following combinations	
a+a	green seedling	a+b, c or d	green seedling
b+b	yellow-green seedling	b+c	green seedling
c+c	pale-yellow seedling	b+d	yellow-green seedling
d+d	white seedling	c+d	pale-yellow seedling

FIG. 2.—**a.** Diagram of the chromatin organization of the end of the short arm of chromosome 9. The large hatched oval represents the terminal heterochromatic knob. This is followed by a thin chromatic segment which joins the first distinct chromomere with the knob. The small, solid ovals represent the two distal chromomeres. The arrow points to the locus of *Yg2*. **b.** Same as **a**, except that the chromosome carries the locus of *yg2* (arrow)' **c.** The end of the short arm of a chromosome 9 deficient for the knob and the segment which joins the knob with the distal chromomere. The locus of *Yg2* is marked by the arrow. This deficiency, when homozygous, gives rise to the pale-yellow seedling phenotype. **d.** Slightly longer terminal deficiency than in **c**. The locus of *Yg2* has been lost. This deficiency, when homozygous, gives rise to the white-seedling phenotype.

The mutants *yg2*, *pyd* and *wd* give rise to peculiar allelic relationships which might be difficult to interpret were the cytology not known. With regard to dominance, there are two series of descending order: I, green→*pyd*→*wd* and II, green→*yg2*→*wd*. The white mutants are common to both series but the *pyd* mutants and *yg2* are not allelic.

THE RATE OF PRODUCTION OF THE *pyd* AND *wd* MUTANTS BY RECENTLY BROKEN CHROMOSOMES 9

It was stated in the introduction that the mutants *pyd* and *wd* appeared repeatedly in the progeny of plants which had received a newly broken chromosome 9. As described earlier, each of the seven *pyd* mutants and each of the six *wd* mutants described in this paper arose independently from a chromosome 9 which was first broken at a meiotic anaphase. Large numbers of functional male gametes containing recently broken chromosomes 9 may be obtained by

special methods (McCLINTOCK 1943). Because of the breakage-fusion-bridge cycle which these meiotically broken chromosomes undergo in the succeeding gametophytic mitoses, the gametes carrying recently broken chromosomes 9 have various modifications in the constitution of the short arm (see page 480). To obtain some estimate of the proportion of functional male gametes which introduce into the embryo the deficiencies responsible for pale-yellow or white seedlings, the following experiment was performed. The silks of plants that were heterozygous for the longer terminal deficiencies of table 4 (that is, deficient for four or six terminal chromomeres) were pollinated by plants that are producing meiotically broken chromosomes 9. The gametophytes produced by the female parent are of two types, those possessing a normal chromosome 9 and those possessing a long terminal deficiency of the short arm of chromosome 9. Whenever male gametes with recently broken chromosomes 9 are delivered by pollen tubes to these female gametophytes, kernels with morphologically normal endosperms will be produced when the female gametophyte possesses the normal chromosome 9. In contrast, aberrant endosperms will be produced when the female gametophyte possesses the deficient chromosome 9. This is due to the subsequent behavior of the broken chromosome 9 delivered to the endosperm by the male parent. It undergoes the breakage-fusion-bridge cycle (McCLINTOCK 1941a) during endosperm development. This process brings about deletions of segments of the short arm of this chromosome 9 in some cells during endosperm development. Since the chromosomes 9 delivered by the female parent are already deficient for a long terminal segment, the telophase nucleus which receives this newly broken chromosome with a terminal deficiency will be homozygous deficient for a segment of the short arm of chromosome 9. In these nuclei, the extent of the homozygous deficiency may range from minute to the full extent of the deficiency in the chromosomes 9 delivered by the female parent. All of these homozygous deficient cells are viable and capable of multiplication. Cells with the longer homozygous deficiencies produce sectors within the endosperm which are sufficiently aberrant to be readily recognizable (McCLINTOCK 1942). Thus, kernels receiving deficient chromosomes 9 from the female parent and a recently broken chromosome 9 from the male parent may be readily detected and selected from an ear. The embryos of these kernels will have the deficient chromosome delivered by the female parent and the newly broken chromosome delivered by the male parent with the exception of a few cases where hetero-fertilization may have occurred. The chromatid type of breakage-fusion-bridge cycle, which occurs in the gametophyte and endosperm tissues, usually does not occur in the sporophytic tissues. The broken end usually heals in the very young embryo and the broken chromosome is completely normal in its mitotic behavior from then on. If the healed broken chromosome 9 has at least a full genic complement of the short arm of chromosome 9, green seedlings should arise from the embryos of these kernels. If it has a short terminal deficiency either pale-yellow or white seedlings could appear because the cells would be homozygous deficient for the short terminal deficiency. If it has a terminal deficiency much beyond the ex-

tent of the *wd* mutants described in this paper, the embryos are expected to be inviable.

From the cross just described, 3287 seedlings were obtained from kernels classified as having received a deficient chromosome from the female parent and a newly broken chromosome from the male parent. Of these seedlings, 77 were pale-yellow and 48 were white. From these results it is concluded that among the viable zygotic combinations, one recently broken chromosome in every 26 had either a deficiency which produced *pyd* or a deficiency which produced *wd*.

These results, together with those already presented for the seven *pyd* and six *wd* mutants described in the previous sections of this paper, illustrate the repeated occurrence of phenotypically and genetically similar mutants. The described chromosomal breakage mechanism is, then, a "mutation inducing" process which "induces" the same mutation time and again.

DISCUSSION

Evidence that some recessive mutations are the consequency of homozygous minute deficiencies has been accumulating in both Drosophila and maize. In Drosophila, the phenotypic characteristics of *y* (EPHRUSSI 1934; STERN 1935; MULLER 1935; DEMEREC 1936; DEMEREC and HOOVER 1936), *sc* (STURTEVANT and BEADLE 1936), *ac* (MULLER 1935), *rst*^{2} (EMMENS 1937; PROKOFYEVA-BELGOVSKAYA 1939; PANSHIN 1941), *w* (PANSHIN 1938, 1941) and possibly *fa* (OLIVER 1937, 1938) in the X chromosome may appear when the + locus of these mutants are missing from the chromosome, that is, when the organism is homozygous deficient, in each case, for a particular minute segment of chromosome. In maize, the appearance of white seedlings as the consequence of a homozygous deficiency of the tip of the short arm of chromosome 9 was first observed by CREIGHTON (1937). This deficiency was internal in that only the proximal part of the knob was included in the deficiency. This deficiency was very minute and it probably included the same segment of chromatin that is responsible for the white seedling phenotypes described in this paper. This deficiency was male and female transmissible and produced white seedlings when homozygous. When combined with *yg2*, the yellow-green phenotype appeared, indicating that the locus of *Yg2* had been included in the deficiency. The genetic behavior of this cytologically similar deficiency duplicated the behavior of the deficiencies causing the white seedlings described in this paper. However, because this stock has been lost, a test for identity could not be made.

A series of recessive mutants associated with homozygous minute deficiencies confined within the limits of a few chromomeres adjacent to the centromere of the short arm of chromosome 5 in maize has been reported previously (MCCLINTOCK 1941b). One of these deficiencies resembled in all ways and was isoallelic if not identical to a previously known recessive mutant (*bm1*) which has been located within this segment. It seems reasonable to conclude that one form of mutation is related to loss of a particular minute segment of

chromatin or to the inactivation of this particular minute segment. The same character could appear following either condition. However, reverse mutation would not be anticipated following loss of a locus, whereas such a reverse mutation might occur following inactivation of a locus. The *y* locus in the X chromosome of Drosophila may illustrate this distinction. Some mutations to *y* may be the consequence of a minute chromatin loss. Other mutations to *y* may be due to inactivations for reversions from *y* to y^+ have been reported (JOHNSTON and WINCHESTER 1934; DUBININ and GOLDAT 1936).

With so few analysed cases available, it is difficult to ascertain the role that homozygous minute deficiencies or inactivations play in the whole mutation process. It seems reasonable to believe that they may play a large part in maize. Within the confines of four chromomeres adjacent to the centromere of the short arm of chromosome 5, six distinct non-allelic mutants, five of which were color mutants and one of which was a developmental mutant, were distinguished. All these mutants were associated with homozygous minute deficiencies. All were both male and female transmissible (MCCLINTOCK 1941b and unpublished). Again, in this paper, mutants associated with minute losses of chromatin have been described. These, too, are both male and female transmissible. Since a color change was the factor which made most of these mutants readily recognizable, it is reasonable to conclude that other mutants, not associated with color changes, are being produced as the consequency of homozygous minute deficiencies. There is no reason to believe that the two chromosome regions in maize which have been selected for study are exceptional samples of the whole chromosomal complement. Their selection was merely a matter of chance because of structural abnormalities that had happened to these chromosomes. It was these structural abnormalities that furnished the means for a study of homozygous minute deficiencies.

In this paper, it has been stated that the recessive mutants pale-yellow and white were due to progressive losses of chromatin. The *pyd* mutants appeared when the chromatin between the knob and the first distinct chromomere was missing and the *wd* mutants appeared when this segment plus an adjacent segment of the first chromomere was missing. The author does not believe that this indicates that the phenotypes pale-yellow and white are due to cumulative effects of the losses described. It is possible that the pale-yellow phenotype is related to loss of a particular locus in the proximal region of the segment which is missing; and that the white phenotype represents the effect of this particular loss plus loss of another particular locus in the adjacent chromomere or loss of only a single locus in this chromomere. The fact that other chromatin is also missing in each case may have little or no relation to the phenotypic expressions of pale-yellow or white. A suggestion that the particular phenotypic characters pale-yellow and white may be due to losses of specific loci rather then cumulative effects of a series of loci, may be seen in the differences between *wd*1, 3, 4, 5 and *wd*2 and 6 in the transmission of the deficient chromosome through the pollen. The four white seedling mutants in the former case have normal transmissions of the deficient chromosomes whereas the latter

two white seedling mutants have a reduced transmission through the pollen. It is possible that a slightly longer deficiency is present in *wd2* and 6. However, when homozygous, this added deficiency does not affect the expression of the white seedling phenotype. The white seedling mutants are semi-dwarfed. This reduced growth rate may be due either to a cumulative effect of various homozygous deficient loci or to a specific locus which is not related to the locus whose absence is responsible for the chlorophyll abnormality. Similarly, death of some of the homozygous deficient embryos in the white seedling cultures may be a reflection of the same phenomenon. Internal deficiencies of specific loci within this segment are required to differentiate between these alternatives. Cytologically, it might be difficult to identify such minute internal deficiencies. In this study, it was only because the segments were terminal that it was possible to analyse the extent of the minute deficiencies with any reasonable degree of certainty. If these deficiencies had been internal, a positive conclusion might not have been obtained. This is because, following homologous association of a normal and a deficient chromosome, the chromomeres adjacent to the internal deficiency might frequently be stretched and distorted during the preparation of the sporocytes for microscopic observation. The sporocytes in pachytene are gently pressed to flatten the chromosomes. When no structural heterozygosity is present, homologous chromomeres remain together during this process. If, however, a small internal deficiency were present in one chromosome, the corresponding non-deficient segment in the homologous chromosome might be subject to tension while being flattened. This tension could result in distortion of the form of the chromomeres adjacent to the deficiency in the deficient chromosomes. This would cause difficulty in the determination of the extent of a very small internal deficiency. When the deficiency is terminal, the free ends of the synapsed chromosomes are not subject to this type of distortion so that small terminal deficiencies may be satisfactorily analysed.

In Drosophila, mutations may be associated with homozygous deficiencies, with duplications, with various "position effects," or dominant mutants may appear when various regions of the chromosome are hemizygous (the Minutes, Notchs, etc.). These mutants are not considered as having arisen solely from modifications of a specific locus—a "genic change." When a mutation arises which is not associated with a visible change in a chromosome, it is not possible with our present methods to know whether a minute deficiency or duplication is present, whether inactivation or a molecular change in a so-called gene has occurred, or whether structural alterations giving "position effects" have occurred. This applies to the majority of mutants that have been studied. From the accumulating evidence in maize and Drosophila, it is conceivable that many of these mutants are not caused by "genic changes," if this is construed to mean a molecular change in an isolated unit. It appears to the author that the interchangeable use of the terms "mutant" and "gene" should be avoided in order not to prejudice ones thinking of genic action.

Just as mutations are not always caused by "genic changes" at a specific

locus, so are alleles not always caused by "genic changes" at a specific locus. In this paper, pale-yellow and white behave as alleles and white and yellow-green behave as alleles but pale-yellow and yellow-green do not behave as alleles.The interpretation given in this paper adequately accounts for these allelic expressions. It is not necessary to invoke a "genic change." Whether or not allelic expressions for specific mutants will occur may depend upon the particular modification which gave rise to the mutation in each case. It is possible that the *pyd* mutant is due to a loss of a specific locus and that *wd* is due to loss of another nearby but independent locus, and also *yg2* may be caused by loss of still another independent locus. If this is true, it should be possible to obtain a chromosome with only the *Pyd* locus missing and also a chromosome with only the *Wd* locus missing. No allelic expressions of *pyd* and *wd* or of *yg2* and *wd* would be anticipated following combinations of these chromosomes. It is only because the *pyd* and *wd* mutants described in this paper have relatively large segments of chromatin missing that we are certain to obtain residual deficiencies and thus allelic expressions following combinations of these deficient chromosomes. Thus, whether or not two or more mutants will show allelic relationships may depend upon the particular modification which gave rise to the mutant. Following some modifications, two mutants, *a* and *b*, may show allelic expressions. If, following another modification, the *a* mutant phenotype arises again, this mutant may show an allelic expression with the original *a* mutant but need not show an allelic expression with *b*. Alleles in the Truncate series, the vestigial series and the facet-Notch series in Drosophila may illustrate such variations in allelic expressions.

In Drosophila, the allelic expression of the *sc* (scute) series (for extensive literature citations see GOLDSCHMIDT (1938), resembles the allelic expressions of *pyd*, *wd* and *yg2*. Overlapping and residual effects follow combinations of specific alleles. This similarity in allelic expression, however, does not presuppose a similarity in cause. The mutants lz^s (spectacle) and lz^g (glassy) behave as alleles but this allelic expression disappears following specific types of crossing over (OLIVER 1940, 1941). Likewise, in Drosophila, the mutants *S* (dominant star) and *ast* (recessive asteroid), which are 0.02 crossover units apart behave as alleles when carried by opposite chromosomes but this allelic expression disappears when both mutants are carried by the same chromosome (LEWIS 1941, 1942). Also, in Drosophila, a deficiency of a particular segment of a chromosome may give rise to a dominant (homozygous lethal) mutant which shows some resemblance to a recessive mutant whose locus is in the deficient segment. When the deficient chromosome and a chromosome with the recessive mutant are combined, the phenotypic expression may be exaggerated form of the recessive mutant. According to BRIDGES (MORGAN, BRIDGES and SCHULTZ 1938), these mutants are "pseudo-allelic." The term "pseudo-allelic" presupposes a knowledge of some special alteration which accompanies the expression of allelism. When this knowledge is not present no "pseudo" modifies the term "allelic." Various causal factors produce mutations and are responsible for allelic expressions. Mutants giving allelic expressions need not be "located"

at comparable positions in homologous chromosomes and they need not be inseparable by crossing-over. It has been the purpose of this paper to analyze one type of modification which gives rise to mutants that show allelic expressions.

The induction of mutations by various means (X-rays, neutrons, U.V. light, heat, age, moisture content of seeds, etc.) has occupied the attention of many geneticists and has proven highly effective. By none of these agents, however, has it been possible to control the particular mutation which will appear. To this list one can add the method described in this paper which involves the repeated occurrence of breaks that are confined within the limits of a single arm of a particular chromosome. Literally thousands of such newly arising broken chromosomes can be obtained with extraordinarily little effort. Since the chromosome arm involved is relatively short, there is a good chance that among a large number of such breakages, many will occur at approximately the same position. In other words, terminal deficiencies of approximately the same length could repeatedly be produced. It has been shown in this paper that the mutants *pyd* and *wd* are associated with such terminal deficiencies. Thus, the mutants *pyd* and *wd* should appear repeatedly in the progeny of plants receiving such newly broken chromosomes. That this is true, has been demonstrated. This broken chromosome method of mutation induction differs from the agents mentioned above in that it repeatedly produces the same mutants. In this respect, it simulates the behavior of the *Dt* mutant in maize which repeatedly induces mutations at a particular locus in another chromosome (RHOADES 1938). However, the mutation process in the two cases is altogether different.

SUMMARY

A number of individuals were obtained possessing a normal chromosome 9 and a chromosome 9 whose short arm was deficient for a terminal segment of chromatin. In each plant, the deficient chromosome was introduced by one parental gamete following breakage of the short arm of chromosome 9 in the previous meiotic mitosis of this parent. The extent of these deficiencies ranged from minute to the full short arm. The smaller terminal deficiencies were both male and female transmissible. Self-pollination of plants heterozygous for these smaller terminal deficiencies gave rise to kernels with homozygous deficient endosperms and embryos. In any one progeny, the seedlings arising from these kernels were either pale-yellow or white.

Seven of these cultures which segregated pale-yellow seedlings were selected for study. In each case, it was determined that the pale-yellow phenotype was produced when the seedlings were homozygous deficient for a minute terminal segment. The seven pale-yellow mutants were comparable in all ways to typical recessive mutants. All seven independently arising pale-yellow mutants were allelic.

Six cultures which segregated white seedlings were selected for study. In all six cases, it was shown that the white seedling phenotype appeared when the seedlings were homozygous for the deficiency. All six white seedling mutants

were allelic. The terminal deficiencies producing the white phenotype are slightly longer than those producing the pale-yellow phenotype.

Intercrosses of the seven pale-yellow mutants with the six white mutants showed that the two types of mutants were allelic. The pale-yellow mutants were dominant to the white mutants. This could be expected, for the individuals possessing a pale-yellow producing deficiency and a white producing deficiency are homozygous deficient for only the shorter of the two deficiencies, that is, the deficiency which produces pale-yellow.

The seven pale-yellow mutants and the six white mutants were combined with a previously isolated recessive mutant yellow-green 2 (*yg2*) known to be located near the end of the short arm of chromosome 9. The seven pale-yellow mutants were not allelic to *yg2* but all six white mutants were allelic and recessive to *yg2*. The allelic expressions of pale-yellow and white, of white and *yg2* and the nonallelic expression of pale-yellow and *yg2* are readily interpretable if it is assumed that the longer deficiency, which produces the white phenotype, included the locus of *Yg2* whereas the shorter deficiency, which produces pale-yellow, does not extend to this locus.

The method of origin of these terminal deficiencies in the short arm of chromosome 9 is relatively simple. Large numbers of newly derived deficiencies may readily be obtained. Many of these should be of approximately the same length. Since the mutants pale-yellow and white are due to specific deficiencies, these same mutants should appear repeatedly in the progeny of individuals that receive these newly derived deficient chromosomes. Special tests, conducted to determine this, confirmed this expectation.

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